CLAIMS

1. A method of determining the binding site specificity of an analyte that binds to a ligand having at least two different binding sites, which method comprises:

immobilizing the ligand to a solid support,

providing a reference analyte which binds reversibly to the ligand at a binding site thereof and whose dissociation phase after interaction with the ligand is either substantially faster or substantially slower than that of the analyte,

contacting a mixture of the analyte and the reference analyte with the immobilized ligand to permit association to the ligand, the concentration of the one of the analyte and the reference analyte that has the faster dissociation phase being sufficient to at least substantially inhibit binding to the ligand of the one having the slower dissociation phase, should the analyte and the reference analyte both bind to the same binding site on the ligand,

stopping the contacting of the analyte and the reference analyte with the ligand to permit dissociation therefrom,

determining a dissociation-related value for the mixture at at least one pre-selected time during the dissociation phase, and

determining from the determined dissociation-related value or values for the mixture if the contribution to this value or values from the one of the analyte and the reference analyte that has the slower dissociation phase is suppressed, substantial suppression indicating that the analyte and the reference analyte bind to the same binding site, and substantial absence of suppression indicating that the analyte and the reference analyte bind to different binding sites.

2. The method according to claim 1, wherein the reference analyte binds to a known binding site of the ligand.

3. The method according to claim 1, wherein the dissociation-related value or values comprise the degree of dissociation at at least one predetermined time during the dissociation phase.

4. The method according to claim 1, wherein the dissociation-related value or values comprise the variation of the degree of dissociation with time during the dissociation phase or a part thereof.

5. The method according to claim 1, wherein the reference analyte has a faster dissociation phase than that of the analyte.

6. The method according to claim 5, wherein the association and dissociation phases of the reference analyte are represented by a square wave type binding curve, and the association and dissociation phases of the analyte are represented by a binding curve having visible association and dissociation phases.

7. The method according to claim 1, wherein the reference analyte has a slower dissociation phase than that of the analyte.

8. The method according to claim 7, wherein the association and dissociation phases of the analyte are represented by a square wave type binding curve, and the association and dissociation phases of the reference analyte are represented by a binding curve having visible association and dissociation phases.

9. The method according to claim 1, wherein the concentration of the one of the analyte and the reference analyte having the slower dissociation phase is kept constant and the concentration of the one having the faster dissociation phase is successively increased, and the influence of the increase on the dissociation phase of the mixture is determined.

10. The method according to claim 1, wherein the method is repeated with at least one other reference analyte that binds specifically to a different binding site on the ligand.

11. The method according to claim 1, wherein the solid support comprises at least one sensing surface of a biosensor.

12. The method according claim 11, wherein the biosensor is an optical biosensor, preferably based on evanescent wave sensing, for example, surface plasmon resonance (SPR).

13. The method according claim 11, wherein the biosensor is based on evanescent wave sensing.

14. The method according claim 11, wherein the biosensor is based on surface plasmon resonance (SPR).

15. The method according to claim 1, wherein the analyte and each reference analyte are contacted with the sensing surface in a flow cell.

16. The method according to claim 1, wherein the ligand is serum albumin, preferably human serum albumin (HSA).

17. The method according to claim 1, wherein the ligand is a protein kinase.

18. The method according to claim 1, wherein the ligand is a drug target.

19. The method according to claim 1, wherein the method is computer implemented.

20. A kit for carrying out the method according to claim 1, comprising at least one reference analyte capable of binding specifically in a reversible defined manner to a defined binding site of a ligand with multiple binding sites.

21. The kit according to claim 20, which comprises at least two different reference analytes, each having a known binding specificity to a different binding site on the ligand.

22. The kit according to claim 20, which comprises at least one reference analyte whose interaction with the ligand can be represented by a binding curve having visible association and dissociation phases.

23. The kit according to claim 20, which comprises at least one reference analyte whose interaction with the ligand can be represented by a binding curve having a square wave type shape.

24. The kit according to claim 20, which comprises, for at least one binding site on the ligand, at least one reference analyte whose interaction with the ligand can be represented by a binding curve having visible association and dissociation phases, and at least one refererence analyte whose interaction with the ligand can be represented by a binding curve having a square wave type shape.

25. The kit according to claim 20, which comprises a plurality of pairs of reference analytes, each pair binding to a different binding site on the ligand and comprising (i) a reference analyte whose interaction with the ligand can be represented by a binding curve having visible association and dissociation phases, and (ii) a refererence analyte whose interaction with the ligand can be represented by a binding curve having a square wave type shape.

26. The kit according to claim 20, wherein the ligand is a biomolecule.

27. The kit according to claim 26, wherein the biomolecule is a protein kinase.

28. The kit according to claim 26, wherein the biomolecule is drug target.

29. The kit according to claim 26, wherein the biomolecule is human serum albumin (HSA).

30. The kit according to claim 20, which further includes a computer program product comprising computer code means for carrying out the method according to claim 1.

31. An analytical system for detecting molecular binding interactions, comprising:

a sensor device comprising at least one sensing surface, detection means for detecting molecular interactions at the at least one sensing surface, and means for producing detection data which represent the progress of each interaction with time, and

data processing means for performing the binding site specificity determination of claim 1.

32. A computer program comprising program code means for performing the binding site specificity determination of claim 1 when the program is run on a computer.

33. A computer program product comprising program code means stored on a computer readable medium or carried on an electrical or optical signal for performing the binding site specificity determination of claim 1 when the program is run on a computer.

34. A computer system containing a program for performing the binding site specificity determination of claim 1.